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Evaluation of DNA typing as a positive identification method for soft and hard tissues immersed in strong acids

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ABSTRACT

Identification of human remains can be hindered by several factors (e.g., traumatic mutilation, carbonization or decomposition). Moreover, in some criminal cases, offenders may purposely adopt various expedients to thwart the victim's identification, including the dissolution of body tissues by the use of corrosive reagents, as repeatedly reported in the past for mafia-related murders.

By means of an animal model, namely porcine samples, we evaluated standard DNA typing as a method for identifying soft (muscle) and hard (bone and teeth) tissues immersed in strong acids (hydrochloric, nitric and sulfuric acid) or in mixtures of acids (aqua regia). Samples were tested at different time intervals, ranging between 2 and 6 h (soft tissues) and 2–28 days (hard tissues). It was shown that, in every type of acid, complete degradation of the DNA extracted from soft tissues preceded tissue dissolution and could be observed within 4 h of immersion. Conversely, high molecular weight DNA amenable to STR analysis could be isolated from hard tissues as long as cortical bone fragments were still present (28 days for sulfuric acid, 7 days for nitric acid, 2 days for hydrochloric acid and aqua regia), or the integrity of the dental pulp chamber was preserved (7 days, in sulfuric acid only).

The results indicate that DNA profiling of acid-treated body parts (in particular, cortical bone) is still feasible at advanced stages of corrosion, even when the morphological methods used in forensic anthropology and odontology can no longer be applied for identification purposes.

1. Introduction

Rapid and unequivocal identification of human remains is essential, regardless of whether these rests are found at a crime scene, in the wake of a mass disaster or under other circumstances. In such cases, however, several factors (including severe body mutilation, carbonization or decomposition) can prevent anecdotal identification, commonly obtained through visual recognition, or from the inspection of personal artifacts. Successful demonstration of individualization must therefore rely on a combination of positive identification methods, including fingerprint collection and comparison, DNA profiling, forensic anthropology, and forensic odontology.

Furthermore, in certain situations, criminals may purposely attempt to hinder the victim's identification by means of "defensive" mutilation including amputation of the hands (so as not to allow fingerprint comparisons) or disfigurement of the face [1], burning of the body [2], and even dissolution in various household chemicals [3]. In particular, the use of strong acids has reportedly been adopted by the Italian Mafia both as a ritual method for killing the relatives of associates who decided to collaborate with the judicial system and as an effective way to dispose of the victims' corpses [4].

Nevertheless, the kinetics by which strong acids attack soft and hard tissues and, consequently, can interfere with the different possible identification strategies has been studied very limitedly so far. Since dental enamel is the strongest component of the human skeleton, and teeth can therefore withstand severe physical and chemical injuries still allowing a comparison with antemortem records, most studies have focused on the effects of corrosive products on human dentition. In particular, Mazza et al. [5] were the first to note that isolated teeth were completely dissolved after 12–17 h of immersion in hydrochloric acid, nitric acid or aqua regia (i.e., a mixture of nitric and hydrochloric acid in 1:3 volume ratio), while the destruction of teeth samples immersed in sulfuric acid was still incomplete after 90 h. Further studies involving the use

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of either pure solutions or household products containing sulfuric, hydrochloric and nitric acid [6–10] gave consistent results and generally indicated that teeth were fully destroyed in nitric and hydrochloric acid within 24 h, whereas sulfuric acid digestion required more time (i.e., between 97 h [10] and 240 h [8]).

Hartnett et al. [8] also tested the effects of common household chemicals, including hydrochloric and sulfuric acid, on human tissues other than teeth (bone, hair, fingernails, and skin/muscle/fat). They observed that hydrochloric acid completely destroyed all biological tissue samples in 24 h or less, with the partial exception of nails and hair (which were reduced to "stubble-like" pieces). With reference to the samples immersed in sulfuric acid, bone was completely dissolved in 6–7 days, while hair and soft tissues took less than 5 h.

All the aforementioned studies, however, have addressed the effects of the acid treatment of soft and hard tissues with an almost exclusive reference to anthropological and odontological identification methods. Only Sowmya et al. [10] used spectrophotometric methods to evaluate the characteristics of DNA isolated from human teeth that had been immersed in hydrochloric acid for 1–5 h. In their study, they described a time-dependent decrease in DNA amount and purity, but they did not perform any further experiment to assess the occurrence of DNA degradation in the tested samples and the feasibility of PCR typing on DNA extracts.

The aims of the present study are therefore (i) to thoroughly evaluate, by means of an animal model, the integrity of DNA isolated from soft and hard tissues exposed to strong acids for variable periods of time, and (ii) to test its suitability for identification purposes, through conventional PCR-based assays designed for the amplification of Short Tandem Repeat (STR) loci.

2. Materials and methods

2.1. Acid immersion experiments

The following strong acids and mixtures of acids were tested: nitric acid ACS reagent, 70% (Sigma–Aldrich, St. Louis, MO, USA); sulfuric acid ACS reagent, 95.0–98.0% (Sigma–Aldrich); hydrochloric acid ACS reagent, 37%, (Sigma–Aldrich); aqua regia (freshly prepared by mixing concentrated nitric acid and hydrochloric acid in a volume ratio of 1:3). The effects of the acid treatment of different tissue types were evaluated by submerging the experimental samples in glass beakers filled with 700–800 ml of acid. Experimental samples were obtained from pigs slaughtered for meat production at a local abattoir and from hunted wild boars subjected to routine veterinary control. Soft tissue samples ($n = 4$ for each acid) consisted of pork meat specimens (600–800 g) that included skin, muscle, fascia, and connective tissue. As for bone tissues, sections of adult pig femurs were used ($n = 4$ for each acid), obtained cross-sectioning each bone at the middle of the diaphysis. In submersion experiments an acid-resistant plastic cap was placed on top of the diaphysis, so as to prevent direct contact between the open medullary cavity and the acid solutions (Supplementary Fig. 1). To simulate the position of the teeth within the mouth, mandibles/maxillae were resected from adult wild boar carcasses with a Stryker autopsy saw, and placed in each type of acid bath ($n = 4$ for each acid). Saw cuts were made high on the rami (mandible) and at the piriform aperture (maxilla) to avoid possible damage to third molars and apices of maxillary teeth.

2.2. DNA extraction

After immersion, soft tissue specimens were harvested at regular intervals of 2 h until they were completely dissolved. At each interval, two 25 mg tissue samples were collected from each specimen, one at its surface and one from a deeper area not in direct contact with the acid solution. After rinsing in water, DNA was extracted from the collected samples with the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Bone and teeth samples were harvested at 2-, 7-, and 28-day intervals. Bone fragments were rinsed in water and air-dried; subsequently, 0.5–2 g of cortical bone tissue was scraped off from the diaphysis with a rasp and manually grinded in liquid nitrogen. The bone powder was demineralized by incubation in 8 ml of EDTA 0.5 M pH 8.0 (Sigma–Aldrich) under shaking at room temperature for 24–48 h; it was subsequently placed in an agitated water bath at 37 °C for 48 h and digested in 7.5 ml of lysis buffer consisting of EDTA 0.5 M pH 8.0 (Sigma–Aldrich), TRIS–HCl 0.05 M pH 8.0 (Sigma–Aldrich), sodium dodecyl sulfate 0.5% (Sigma–Aldrich) and 1 mg proteinase K (Macherey-Nagel, Düren, Germany). Lastly, the lysate was extracted with the QIAamp DNA Blood Maxi kit (Qiagen) and concentrated to 50 µl with the QIAamp DNA Micro kit (Qiagen) according to the manufacturer's instructions.

Upper/lower molar teeth, selected on the basis of normal morphology and absence of carious lesions, were extracted from mandibles/maxillae and processed as described by Pinchi et al. [11]: in brief, endodontical access of the pulp chamber provided pulp residues from which DNA was isolated using the QIAamp DNA Micro kit (Qiagen), according to the manufacturer's instructions, in a final elution volume of 50 µl.

DNA reference samples of the same animals from which soft and hard tissue specimens were derived were obtained from 25 mg of untreated muscle tissue extracted with the QIAamp DNA Mini kit (Qiagen).

2.3. DNA quantitation

Quantitation of porcine DNA in the acid-treated specimens was performed by means of a TaqMan quantitative polymerase chain reaction (qPCR) assay targeting the beta-actin gene. The sequence for *sus scrofa* beta-actin gene was retrieved from GenBank (www.ncbi.nlm.nih.gov/genbank). Candidate primer and probe sequences were generated with the Primer Express software (Thermo Fisher Scientific, Waltham, MA, USA) and then selected on the basis of species specificity verified using the Basic Local Alignment Search Tool software (http://blast.ncbi.nlm.nih.gov). Sequence characteristics and concentration of the primers and of the FAM-labeled, Iowa Black-quenched probe (Bio-Rad Laboratories, Hercules, CA, USA) used in the assay are shown in Table 1. qPCR amplification was performed on the CFX96 Real-Time PCR system (Bio-Rad Laboratories) in a final volume of 10 µl including 1× SsoAdvanced Universal Probes Supermix (Bio-Rad Laboratories) and 1 µl of template DNA. The PCR protocol consisted of a 3-min pre-PCR heat step at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s, and annealing/extension at 60 °C for 30 s. Experimental samples were quantified by comparison with an eight-point standard curve generated from serial dilutions (1:3) of high-quality porcine DNA (100 ng/µl) isolated from fresh muscle tissue and quantified using the Qubit 2.0 fluorometer (Thermo Fisher Scientific). The standard curve was run in duplicate with every qPCR plate to ensure accurate quantification data.

2.4. DNA typing

Multiplex PCR amplification of eleven STRs and of an Amelogenin-like sex marker (SBH23) was executed using the Animal Type Pig PCR amplification kit (Biotype AG, Dresden, Germany) [12] and 0.5 ng of template DNA, following the manufacturer's recommendations. Typing was performed by capillary

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Table 1
Characteristics and concentrations of primers and probe of the qPCR assay for quantitation of porcine DNA: oligonucleotide base positions are given in accordance to *sus scrofa* reference sequence (NC_010445) deposited in GenBank.

Oligonucleotide	Position	Sequence	Concentration (nM)
Forward primer	4,729,379–4,729,402	TGTCCCGCAACTTGAAGTATGAAG	900
Reverse primer	4,729,339–4,729,355	TGCCTCCGCACCTCAAC	900
Probe	4,729,358–4,729,377	FAM-CTTTTGGTCCCCCTAGGAGC	250

electrophoresis on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) in comparison with allelic ladders and a control DNA sample (DL157) of known genotype provided with the Animal Type Pig PCR amplification kit.

2.5. Statistical analysis

Statistical analysis was performed with SigmaStat v 3.1 software (Systat Software, San Jose, CA, USA). Student's *t*-test (parametric data) and Mann-Whitney *U* test (non-parametric data) were used for testing of statistical significance.

3. Results

3.1. Soft tissues

In agreement with the experiments with sulfuric and hydrochloric acids conducted by Hartnett et al. [8], soft tissues were completely dissolved within 6 to 8 h, regardless of the type of acid that was used. Quantitation by qPCR showed that, among tissue samples collected after 2-, 4- and 6-h intervals, only deep samples from specimens immersed in nitric acid for 2 h still displayed DNA concentrations above the assay's lower limit of detection as determined by the applied standard curve (0.046 ng/μl). In these samples, the average DNA yield per mg of tissue was of 7.4 ng/mg (± 6.0 SD), and a full STR profile consistent with the reference samples was obtained for every tissue replicate ($n = 4$) (a selection of electropherograms is shown in Fig. 1). In all the remaining soft tissue samples, porcine DNA was not detected by qPCR and no STR profile could be obtained. To verify whether these negative results should be attributed to severe template degradation or to inhibitory activity of strong acids affecting qPCR [13], a further quantification experiment was performed. In brief, for each acid-treated soft tissue sample displaying a negative quantitation result ($n = 44$), 1 μl of DNA was mixed with 1 μl of high-quality porcine DNA (2.5 ng/μl) previously quantified by Qubit. Average cycle threshold (Ct) values resulting from qPCR were 27.4 ± 0.2 SD for 1:1 mixtures of acid-treated and high-quality DNA, and 27.1 ± 0.1 SD for eight replicates of pure high-quality DNA. The extremely limited increase in Ct values registered in the presence of acid-treated DNA (average ΔCt value of 0.22) did not appear large enough to explain the complete amplification failure of the experimental samples. The observed results therefore suggested that negative quantitation values could mainly be ascribed to extensive acid-induced degradation of DNA, which produced fragments smaller than the qPCR assay molecular target (67 bp), whereas the effect of PCR inhibition was almost negligible.

3.2. Bone tissues

With reference to bone tissues, Fig. 2 presents a selection of images documenting the inherent immersion study. Largely intact

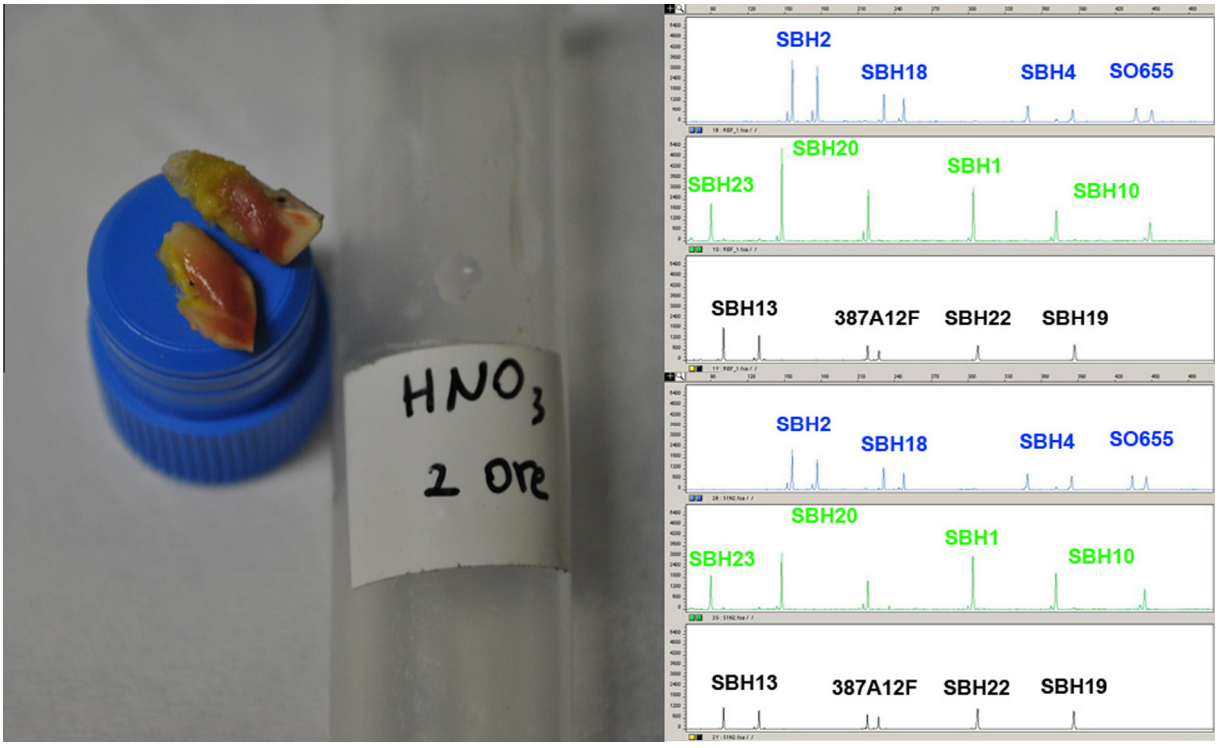

Fig. 1. Soft tissue immersed in nitric acid for 2 h (left): representative Animal Type Pig STR profiles of a treated sample (lower pane) and a reference sample obtained from fresh muscle tissue (upper pane) are shown.

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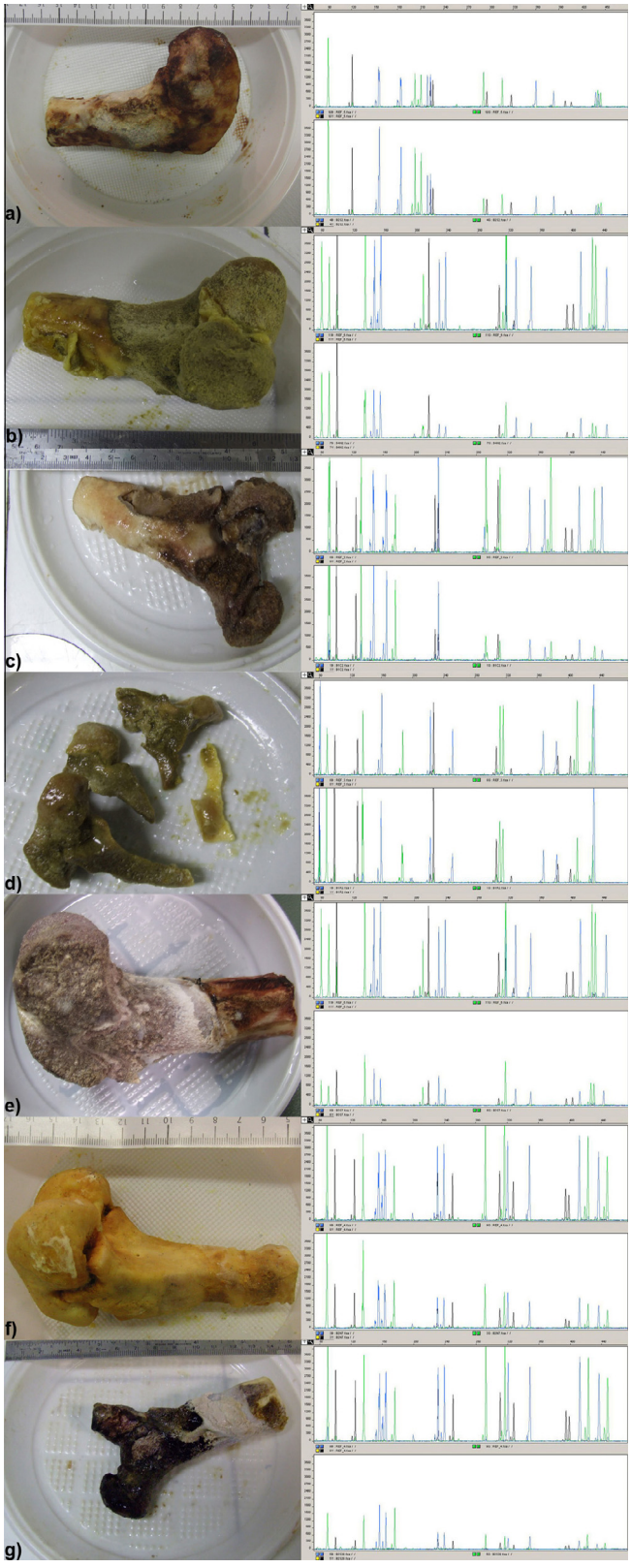

Fig. 2. Pig half femurs after: 2-day submersion in sulfuric acid (a), nitric acid (b), hydrochloric acid (c) and aqua regia (d); 7-day submersion in sulfuric acid (e) and nitric acid (f); 28-day submersion in sulfuric acid (g). For each combination of acid type and submersion interval, representative electropherograms of treated bone samples (upper pane) and reference samples obtained from fresh muscle tissue (lower pane) are shown.

femurs were still present even after 28 days of immersion in sulfuric acid (Fig. 2g). Conversely, bone samples were completely dissolved in hydrochloric acid and in aqua regia between the 2- and the 7-day sampling intervals, and in nitric acid between the 7- and the 28-day sampling intervals, so that no cortical bone suitable for DNA extraction could be retrieved from these experiments. Aqua regia appeared to be the most effective corrosive agent on bone samples, judging from the fact that only few fragments of cortical bone suitable for DNA extraction were found since 2 days after immersion (Fig. 2d).

The results of further qPCR experiments indicated that, regardless of submersion time and acid type, whenever compact bone tissue samples could be retrieved and subjected to DNA extraction (i.e., after 2, 7 and 28 days for sulfuric acid, 2 and 7 days for nitric acid, and 2 days for hydrochloric acid and aqua regia), DNA yields were generally high, to the extent that a 1:20 dilution had to be applied to some extracts not to exceed the upper limit of detection of the qPCR assay (100 ng/μl). DNA yields per mg of bone tissue are summarized in Table 2. Although, as previously mentioned, aqua regia appeared to be the most corrosive agent on bone tissues, the comparison of DNA yields at the 2-day sampling interval demonstrated that hydrochloric acid caused the most intense degradation of nucleic acids. As a matter of fact, a Mann-Whitney U test indicated that samples treated with hydrochloric acid had significantly lower DNA concentrations than all the other tested samples, $U = 10$, $p = 0.01$. Even though a reduction in mean DNA yield could be registered in bone samples immersed in sulfuric acid for 2 to 7 days, such reduction was not statistically significant, $U = 21$, $p = 0.49$. A t-test indicated that DNA yield appeared substantially unchanged after 7 and 28 days of treatment in sulfuric acid $t(6) = 0.18$, $p = 0.86$, and after 2 and 7 days of treatment in nitric acid, $t(6) = 0.32$, $p = 0.76$. In all the tested bone samples, amplification of porcine-specific STRs generated full profiles that were consistent with reference DNA, demonstrating that the extracted DNA was perfectly suitable for forensic purposes (a selection of electropherograms is shown in Fig. 2).

3.3. Teeth

The experiments involving the acid treatment of mandibles and maxillae showed that intact teeth suitable for DNA testing were no longer recognizable after 2 days of submersion (Supplementary Fig. 2), with the only exception of specimens treated with sulfuric acid (Fig. 3). As for sulfuric acid, largely intact mandibles/maxillae could still be retrieved after 7 days of submersion; after 28 days,

Table 2
DNA yields (ng per mg of tissue) obtained from acid treated bone samples: number of replicates for each type of treatment (n), collection time (t) and amount in grams of bone powder used for DNA extraction (g) are given.

Acid type	n	t (days)	g	DNA yield (ng/mg)	
				Range	Average (±SD)
Sulfuric	4	2	2.5	1.0–30.6	15.4 (±14.5)
Sulfuric	4	7	2.5	0.5–16.4	7.3 (±7.8)
Sulfuric	4	28	1.5	2.0–14.1	6.4 (±5.6)
Nitric	4	2	1	1.1–4.5	2.3 (±1.5)
Nitric	4	7	0.5	1.5–2.5	2.0 (±0.5)
Nitric	4	28	–	–	–
Hydrochloric	4	2	0.5	0.2–0.9	0.5 (±0.3)
Hydrochloric	4	7	–	–	–
Hydrochloric	4	28	–	–	–
Aqua regia	4	2	1	4.1–6.3	4.9 (±1.0)
Aqua regia	4	7	–	–	–
Aqua regia	4	28	–	–	–

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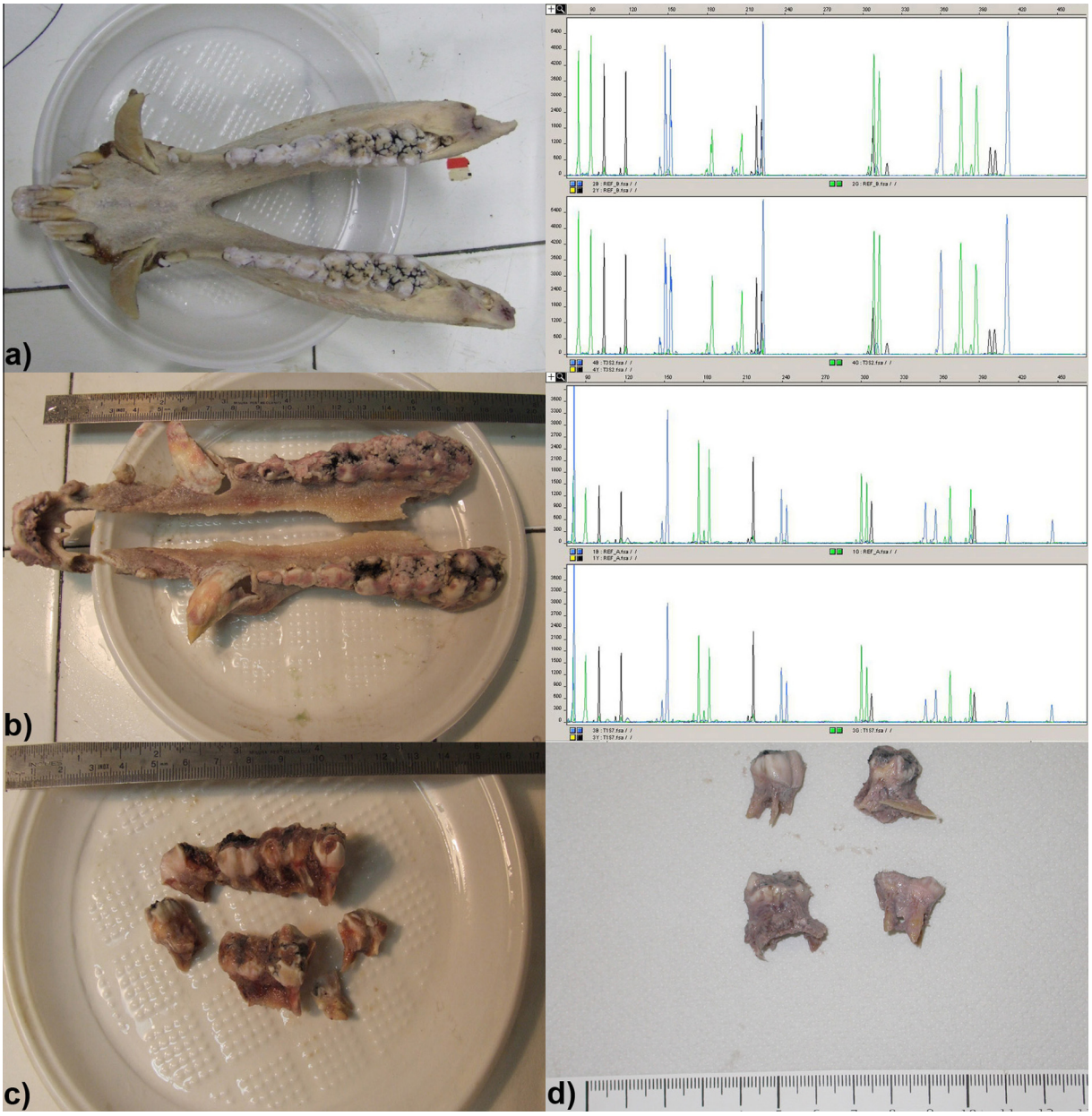

Fig. 3. Wild boar mandibles/maxillae after: 2- (a) 7- (b) and 28-day (c) submersion in sulfuric acid. For 2- and 7-day intervals, representative electropherograms of treated teeth samples (upper pane) and reference samples obtained from fresh muscle tissue (lower pane) are shown. A particular of isolated molar teeth retrieved from sulfuric acid at the 28-day interval, with partially corroded roots, is shown in (d).

instead, only completely isolated teeth and mere fragments of alveolar bone enclosing teeth (with now exposed roots) could be found. In several teeth collected after 28 days of sulfuric acid treatment, the structural integrity of the crown appeared to be preserved, but the exposed roots presented variable signs of acid corrosion (Fig. 3d).

For molars treated with sulfuric acid, DNA yield per tooth widely ranged between 2.9 ng and 1.1 μg (mean 400.0 ng ± 486.5 SD) after 2 days ($n = 4$), and between 60.8 and 376.8 ng (mean 188.6 ng ± 134.2 SD) after 7 days ($n = 4$). A t-test indicated that the decrease in mean DNA yield observed between 2 and 7 days of immersion, however, was not statistically significant, $t(6) = 0.83$, $p = 0.44$. Complete STR profiles consistent with reference DNA were obtained from all the eight samples (a selection of electropherograms is shown in Fig. 3). Conversely, qPCR and STR amplification always displayed negative results for pulp samples obtained from molars ($n = 4$) subjected to 28 days of sulfuric acid treatment. Since only molar teeth with preserved crowns were selected for DNA extraction, it is likely that the penetration of the aforementioned acid through the partially corroded apical foramen and its consequent invasion of the pulp canal and chamber were the cause of rapid and complete DNA degradation, similar to that observed in soft tissues. Inhibition control experiments, performed by qPCR as previously described, were conducted on 1:1 mixtures of high-quality porcine DNA (2.5 ng/μl) and DNA extracted from molar samples immersed for 28 days in sulfuric acid ($n = 4$). An average ΔCt value of only 0.35 was observed between the mixtures (mean Ct 26.5 ± 0.1 SD) and the eight replicates of pure high-quality DNA (mean Ct 26.1 ± 0.2 SD), indicating that severe degradation of nucleic acids, and not a reduction in PCR efficiency,

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was the most relevant reason why DNA and STR profiles had not been detected in teeth specimens after 28-day treatment with sulfuric acid.

4. Discussion

The chemical mechanism underlying acid-dependent tissue damage consists in the release of free hydrogen ions (protons) that catalyses amide bond hydrolysis, which, in turn, causes protein structures to collapse. In addition to protein denaturation, further damage results from both tissue dehydration and extreme heat production when sulfuric and/or hydrochloric acid are used [14]. At molecular level, acid treatment has long been known to produce significant effects on DNA integrity. As a matter of fact, the primary mechanisms involved are depyrimidination and depurination, i.e., the loss of bases by cleavage of the glycosidic bond. At the formed abasic sites, phosphodiester bonds become more susceptible to hydrolysis with subsequent DNA degradation [15]. A similar effect has been described in the literature in relation to acid fixatives such as Bouin's solution, which contains picric and acetic acid [16]. Decalcification agents containing strong acids (e.g., nitric and hydrochloric acid), used prior to routine histological analysis of bone biopsies, have also shown to cause a considerable decrease in both DNA yield and integrity, thereby limiting the suitability of the treated samples for further molecular diagnostic assays [17].

Our experiments confirmed the results of previous reports [8] regarding the ability of strong acids to rapidly dissolve (<8 h) soft tissues. More importantly, total DNA degradation appeared to precede the completion of tissue destruction, occurring after 2 h of immersion even in deep areas of the tested samples not in direct contact with the corrosive agent. Nitric acid was the only exception, with deep samples being amenable to DNA profiling at least at the 2-h interval. This possibly reflects in vivo observations [18], showing a relatively lower capacity of nitric acid to diffuse and penetrate tissue layers, compared with sulfuric and hydrochloric acid.

Although DNA extraction from cementum covering the roots of teeth has been described in the past [19], dental pulp is generally considered the richest source of DNA in teeth [20] on account of its relatively high cellularity. Molars in particular, having the largest pulp volume and root surface area, are considered to be best suited for DNA typing purposes [21], and were specifically targeted in the present study. Highly mineralized enamel and dentine constitute an effective physical barrier that protects dental pulp from environmental degrading agents. However, previous studies on isolated teeth have demonstrated [5–10] that strong acids (especially hydrochloric and nitric acid) can rapidly erode the enamel and expose the dentine surface. In this respect, the results of our immersion experiments indicated that the mechanical protective effect of alveolar bone on teeth was limited in the case of nitric acid, hydrochloric acid, and aqua regia, and that acid digestion times for whole mandibles/maxillae did not significantly diverge from those reported in previous studies in relation to isolated teeth [5–10]. In immersion experiments with nitric acid, hydrochloric acid, and aqua regia, we also noted that the destruction of DNA-rich pulp tissue appeared to immediately follow the erosive process leading to the complete loss of the morphological characteristics of the teeth in less than 48 h. On the contrary, sulfuric acid treatment preserved teeth and surrounding bone structures for a longer time, consequently we observed that DNA typing was still possible at the 7-day interval. Nevertheless, once the erosion of the alveolar bone had exposed the roots and allowed the acid to pass through the pulp canal, complete DNA degradation occurred even in teeth with still intact crowns, as seen at the 28-day interval.

In practice, the time span available for the molecular identification of acid-treated teeth does not exceed significantly that of conventional dental methods. There are, however, several important advantages of DNA technology with respect to odontological identification, including its practicability in the absence of ante-mortem medical or dental records and the opportunity of conducting a thorough statistical evaluation of the evidence. With reference to statistical analysis, it must also be emphasized that immersion in sulfuric acid, even at the 7-day interval, did not seem to affect the quantity and quality of DNA isolated from intact pulp chambers, thus minimizing the risk of partial STR profiles and PCR artifacts due to low template input that could possibly hinder the interpretation of DNA data.

Bone is the hardest tissue in the body after dental enamel and its resistance to morphological degradation both at the macroscopic and microscopic level is the key factor contributing to the protection of DNA from physical, chemical and biological agents. Bone density, which is related to the extent of mineralization, is highest in weight-bearing long bones (e.g., femurs) and, more specifically, at the diaphysis; in this respect, bone composition in pigs closely resembles that found in humans [22]. Our experiments showed a persistence of bone samples in acid environment consistently longer than previously observed by Hartnett et al. [8], who reported a dissolution time of 6–7 days for sulfuric acid, and less than 20 h for hydrochloric acid. The difference can be attributed to the fact that in the aforementioned study one-inch sections of femoral diaphysis were used, instead of half-femurs. Accordingly, Hartnett et al. [8] stated that, when immersing an entire proximal epiphysis in hydrochloric acid, complete destruction was delayed and could not be reached until 23 h. In our study, significantly longer corrosion times for femurs rather than for teeth samples were observed in nitric acid, hydrochloric acid, and aqua regia. After 28 days of immersion in sulfuric acid, fragmented samples of mandibles/maxillae and partly corroded isolated teeth were still present, as well as largely intact femurs, but dental pulp was no longer suitable for DNA typing. On the contrary, the original thickness of femur cortical bone (about 3 mm in adult pigs [23]) enabled part of the osteocyte lacunae and their nuclear DNA content to be preserved from acid aggression, as demonstrated by the high yields of intact DNA obtained even at stages of immersion when only few eroded parts of compact bone could still be retrieved (e.g. after 2-day treatment with hydrochloric acid). Compact bone consistently proved to be the only tissue allowing STR-based identification of the experimental samples at the most advanced stages of corrosion, regardless of the acid type (2 days for hydrochloric acid and aqua regia, 7 days for nitric acid, and 28 days for sulfuric acid). These time intervals always exceeded those reported for teeth samples in the case of both the molecular assays used in the present research (7 days for sulfuric acid only) and the morphological methods evaluated in previous studies (<24 h for hydrochloric and nitric acid, approximately 4 to 10 days for sulfuric acid) [5–10].

Despite some obvious limitations in the experimental setup (e.g. the choice of an animal model and the use of relatively small body parts immersed in limited volumes of acid), the present study clearly demonstrated that positive genetic identification of hard tissues, and of bone samples in particular, is possible after prolonged acid treatment. Complete STR profiles were obtained from femur samples immersed for up to 28 days in sulfuric acid, 7 days in nitric acid, and 2 days in hydrochloric acid and aqua regia. It must also be emphasized that in real-case scenarios, supposing whole corpses or large body parts were dissolved, the reported time spans could even be extended, at least for less corrosive or more diluted acids. For example, we noticed that nitric acid required at least 4 h to penetrate the core of small experimental meat specimens (600–800 g) and completely degrade DNA. Therefore, it can be expected that the physical protection exerted

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by various tissues in whole body parts (above all intact skin and thick muscles, as in the lower limb), will further delay the effects of acids on nucleated cells surrounded by, or embedded in, highly resistant mineralized matrices such as those found in cortical bone and teeth.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.legalmed.2015.07.004.

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